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Cross-Resistance to Imidacloprid in Strains of German Cockroach (*Blattella germanica*) and House Fly (*Musca domestica*)

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Abstract: The toxicity of a promising new insecticide, imidacloprid, was evaluated against several susceptible and resistant strains of German cockroach and house fly. Imidacloprid rapidly immobilized German cockroaches followed by a period of about 72 h during which some cockroaches recovered. After 72 h there was no further recovery. Imidacloprid-treated houseflies were immobilized more slowly than treated cockroaches, with the maximum effect observed after 72 h, and there was no recovery. Based upon 72-h LD_{50} values imidacloprid was moderately toxic to German cockroaches (LD_{50} values were 6–8 ng mg^{-1}) and had only low toxicity to house flies (LD_{50} 140 ng mg^{-1}). Piperonyl butoxide (PBO) blocked the observed recovery in German cockroaches. PBO also greatly enhanced the 72-h LD_{50} of imidacloprid from 43- to 59-fold in cockroaches and 86-fold in house flies.

Two strains of German cockroach (Baygon-R and Pyr-R) showed >4-fold cross-resistance to imidacloprid. This cross-resistance could not be suppressed by PBO, suggesting that P450 monooxygenase-mediated detoxication is not responsible for this cross-resistance. Variation in the level of synergism observed with PBO (between strains) suggests the 'basal' level of monooxygenase-mediated detoxication of imidacloprid is quite variable between strains of German cockroach.

The AVER and LPR strains of house fly showed significant cross-resistance to imidacloprid. PBO reduced the level of cross-resistance in AVER from >4·2-fold to 0·5-fold (i.e. the AVER strain LD_{50} was half that of the susceptible strain when both were treated with PBO), but PBO did not suppress the cross-resistance in LPR. These data suggest monooxygenases are the mechanism responsible for cross-resistance to imidacloprid in AVER, but not in the LPR strain.

Key words: Insecta, imidacloprid, synergism, insecticide

1 INTRODUCTION

German cockroaches (*Blattella germanica* L.) and house flies (*Musca domestica* L.) are two pests of economic and medical importance.[1,2] Insecticides are a major means for controlling these pests. However, the development of resistance and the loss of insecticides due to regulatory actions necessitate the development of safe, new materials for control of these pests.

Imidacloprid is a promising new insecticide that may be of value in the control of German cockroaches and house flies. Imidacloprid exerts its toxic action by interacting directly with the nicotinergic acetylcholine receptor (nAChR).[3–9]

Cross-resistance is a potential problem that could limit the lifetime of a new insecticide. Although cross-

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resistance may not prevent the development of new classes of insecticides (i.e. pyrethroids were successfully developed even though there was cross-resistance in some DDT-resistant strains), such information is important in the development of resistance management strategies for new compounds. Furthermore, cross-resistance studies often reveal useful information about the mechanism of action and metabolic pathways of new insecticides.

In order to evaluate the effectiveness of imidacloprid, and the potential for cross-resistance, we tested this insecticide against susceptible and resistant strains of German cockroach and house fly. The toxicity of imidacloprid was also tested following treatment with the monooxygenase inhibitor piperonyl butoxide (PBO) to evaluate the relative role of cytochrome P450 microsomal monooxygenases in metabolism and cross-resistance.

EXPERIMENTAL

2.1 Insects and chemicals

Nine strains of German cockroach were used: CSMA is an insecticide-susceptible strain obtained from F. Matsumura in 1986, Cochran-S is a susceptible strain obtained from D. Cochran in 1992,[10] Ectiban-R is a pyrethroid-resistant strain selected with permethrin from the DDT-selected VPIDLS strain,[11] Dursban-R, Rutgers and Kenly strains are multi-resistant to several organophosphate and carbamate insecticides,[11,12] Pyr-R was selected from the Kenly strain using pyrethrins,[13] Baygon-R was selected from the Kenly strain using propoxur[14] and Cld-R is a cyclodiene-resistant strain selected from the LPP strain originally obtained from F. Matsumura in 1986. Cockroaches were reared as described by Scott *et al.*[11]

Three strains of house fly were used: CS is an insecticide-susceptible strain,[15] LPR is a multi-resistant strain having high levels of resistance to pyrethroid insecticides[16] and AVER is an abamectin-resistant strain.[17] House flies were reared as described by Wheelock and Scott.[18]

Imidacloprid (97·4%) was supplied by Bayer (Kansas City, Missouri) and piperonyl butoxide (PBO) was from Chemical Dynamics Corporation (South Plainfield, New Jersey).

2.2 Bioassays

Insecticide or PBO was delivered in 0·5 μl acetone to the abdominal sternum of adult male cockroaches[11] or to the thoracic notum of female house flies.[19] PBO was delivered 1 h before the insecticide application at the maximum sublethal dose: 100 μg per cockroach and 10 μg per fly. Ten cockroaches or 20 house flies were treated for each dose. There was a minimum of three doses giving $>0\%$ and $<100\%$ mortality at 72 h after insecticide treatment for all bioassays. Each bioassay was replicated at least three times. The treated insects were put in 200-ml Sweetheart ice-cream cups covered with cheese cloth and maintained at 25°C. Each cup was provided with two pieces of 4-cm dental wick soaked in 15% sugar water and the dental wick was kept wet during the experiment. Insects were observed several times during the day following treatment. Mortality was assessed at 24, 48, 72 and 96 h after insecticide application. Insects that were on their backs and unable to right themselves when disturbed were considered dead. The bioassay data were analysed based on standard probit analysis[20] as adapted to personal computer use.[21]

RESULTS AND DISCUSSION

Imidacloprid was relatively fast-acting against German cockroaches. The maximum effect was seen 2–4 h after treatment. Some cockroaches slowly recovered up to about 72 h, after which time there was no further recovery. The amount of recovery was quite pronounced, with the 1·5-h ED_{50} (data not shown) being approximately 10-fold less than the 72-h LD_{50} (Table 1). PBO blocked this recovery, suggesting that monooxygenase-mediated detoxication was involved in this process. Early symptoms of poisoning included hyper-responsiveness, hyperactivity and tremors of the body and legs, followed by ataxia. After several hours these symptoms gradually subsided and the animals became hyporesponsive and hypoactive by 24 h.

The effect of imidacloprid on house flies was somewhat different from that on cockroaches. Imidacloprid-treated houseflies were immobilized more slowly (compared to cockroaches), with the near-maximum effect seen after 24 h (LD_{50} values were 3700 and 2400 ng per fly, 24 and 72 h after treatment, respectively). There was no recovery. In addition, treated house flies became hyporesponsive, hyporeactive and the majority were unable to fly. House flies did not develop the symptoms associated with the early signs of imidacloprid poisoning in German cockroaches.

The moderate toxicity of imidacloprid to susceptible cockroaches (72-h LD_{50} values were 270–410 ng per cockroach) indicates that this material is similar in toxicity to commonly used carbamate and organophosphate insecticides (e.g. bendiocarb and chlorpyrifos), but is substantially less toxic than most pyrethroids.[11] PBO enhanced the toxicity by 43- to 59-fold suggesting that there is substantial P450 monooxygenase-mediated detoxication of imidacloprid in susceptible strains of German cockroach.

TABLE 1
Toxicity of Imidacloprid ± Piperonyl Butoxide to German Cockroaches and House Flies

Strain	Body Weight (mg) (±SEM)	*Imidacloprid* LD_{50}[a] (95% CI)	Slope (SE)	n	RR[b]	*Imidacloprid + PBO* LD_{50}[a] (95% CI)	Slope (SE)	n	SR[c]	RR[b]
German cockroaches										
CSMA	44·6 (±0·8)[d]	270 (190–380)	1·8 (0·3)	280	—	6·3 (4·5–8·5)	1·3 (0·1)	700	43	—
Cochran-S	51·6 (±0·7)	410 (280–580)	1·5 (0·2)	280	1·5	7·0 (4·0–10·4)	1·5 (0·3)	280	59	1·1
Ectiban-R	46·6 (±0·8)[d]	520 (330–740)	1·5 (0·2)	240	1·9					
Dursban-R	49·2 (±1·1)[d]	590 (390–870)	1·4 (0·2)	240	2·2					
Rutgers	56·6 (±1·3)[d]	580 (390–840)	1·7 (0·2)	240	2·1					
Baygon-R	46·7 (±0·6)	1100 (740–1500)	1·5 (0·2)	280	4·1	100 (70–150)	1·6 (0·2)	400	11	16
Pyr-R	49·8 (±1·0)[d]	1800 (1200–2900)	1·1 (0·2)	280	6·7	80 (58–110)	1·5 (0·2)	440	23	13
Kenly	49·9 (±0·9)[d]	750 (510–1000)	1·7 (0·2)	240	2·8					
Cld-R	44·3 (±0·7)	480 (360–630)	2·0 (0·3)	280	1·8	25 (16–37)	1·7 (0·3)	320	19	4·0
House flies										
CS	16·6 (±0·2)	2400 (1800–3300)	1·0 (0·1)	840	—	28 (22–35)	1·8 (0·2)	880	86	—
LPR	18·6 (±0·2)	$>10^4$		1000	>4·2	720 (560–930)	1·0 (0·1)	1200	>14	26
AVER	15·6 (±0·2)	$>10^4$		1080	>4·2	15 (12–20)	1·5 (0·2)	800	>670	0·5

[a] LD_{50} values in ng per insect 72 h post-treatment.
[b] Resistance Ratio: LD_{50} of resistant strain/LD_{50} of CS (house fly) or CSMA (cockroach) susceptible strain.
[c] Synergism Ratio: LD_{50} of insecticide alone/LD_{50} of insecticide + PBO.
[d] Values from Scott.[13]

Two strains of German cockroach had 4·1 to 6·7-fold levels of cross-resistance to imidacloprid when their LD_{50} values were compared to that of the CSMA susceptible strain: Baygon-R (4·1-fold) and Pyr-R (6·7-fold). PBO was not effective in blocking this cross-resistance, suggesting that P450 monooxygenase-mediated detoxication is not involved. Interestingly, PBO had a smaller effect in the Baygon-R, Pyr-R and Cld-R strains relative to the susceptible strains (leading to an increase in the RR to imidacloprid + PBO compared to imidacloprid alone). Thus, it appears that the 'basal' level of monooxygenase-mediated detoxication is quite variable between strains of German cockroach.

Imidacloprid had low toxicity to house flies with a 72-h LD_{50} of 2400 ng per fly in the CS strain (Table 1). The LPR and AVER strains were significantly cross-resistant to imidacloprid (>4-fold), having only 3% and 30% kill at 10 000 ng per fly, respectively. PBO caused 86-fold synergism of imidacloprid in susceptible house flies suggesting that there is substantial P450 monooxygenase-mediated detoxication in this strain. PBO was highly effective against the LPR and AVER strains, resulting in >14- and >670-fold increases in toxicity, respectively. PBO produced no discernible change in the level of cross-resistance in LPR, suggesting monooxygenases are not a major mechanism of cross-resistance to imidacloprid in this strain. PBO had a dramatic effect on imidacloprid toxicity in AVER, making this strain 2-fold more sensitive than the susceptible CS strain. The high level of synergism seen with PBO in all strains of house fly suggests there is substantial monooxygenase-mediated detoxification of imidacloprid in this species.

Imidacloprid was 18- to 23-fold more toxic to German cockroaches than to house flies. The susceptible strain LD_{50} values were 6·1 and 7·9 ng per mg for cockroaches and 140 ng per mg for house flies. PBO slightly reduced this difference between species (to 12-fold). Therefore, the difference in toxicity of imidacloprid between these species is not entirely due to greater monooxygenase-mediated detoxication in house flies.

Imidacloprid, although not highly toxic to house flies or German cockroaches, can be effective when combined with PBO. Given that pyrethrins have had widespread application for the control of household pests it is possible that imidacloprid may also prove useful in controlling these species. Furthermore, the high degree of PBO synergism seen in house flies and German cockroaches suggests that, if the sites of metabolic attack on the imidacloprid molecule could be modified to prevent metabolic attack (and maintain insecticidal activity), a very potent insecticide could be developed.

Cases of both PBO-suppressible and PBO-non-suppressible cross-resistance to imidacloprid were observed. PBO was able to overcome cross-resistance to imidacloprid in AVER house flies, while PBO was without substantial effect in German cockroaches or LPR house flies. Thus, there appears to be more than one mechanism that can confer cross-resistance to imidacloprid. While monooxygenase-mediated detoxication is clearly implicated as the mechanism of cross-resistance in AVER house flies, the mechanism(s) of cross-resistance in the Baygon-R and Pyr-R strains of German cockroach and the LPR strain of fly cannot be deduced from this study.

Recently, a multi-resistant strain of small brown planthopper, *Laodelphax striatellus* (Fall.), was shown to be 18-fold resistant to imidacloprid by topical application.[22] This resistant strain could be controlled by imidacloprid at field application rates. Whether or not the levels of cross-resistance reported here for German cockroaches and house flies would give rise to control problems under field conditions is unknown and will require further study.

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